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IN VITRO MUTAGENESIS OF THREE CULTIVARS OF POTATO BY SODIUM AZIDE

Luma Ali Hussain[1], Falah Hassan Almiahy[2]* and Zainb Abdul- Jabbar[3]

[1,2] Department of Horticulture and landscape, Collage of Agriculture and Marshes, University of Thi-Qar, Thi-Qar,64001, Iraq

[3] Department of Genetic Engineering, Agriculture Research Directorate, Ministry of Science and Technology, P.O. Box 765, Baghdad-Iraq

*Corresponding author: E-mail: falah-ha@utq.edu.iq

Abstract

In vitro of three potato cultivars (Arnova, Provento and Emma) were mutated with sodium azide (NaN3) at a concentration of 1mM for different periods of time (0, 5, 10, 15, 30) minutes. The optimum mutagenic dose was determined and the effect of sodium azide was studied on the phenotypic traits of vegetative (plant height, number of shoots, number of leaves and nodes) and root systems (number and height of roots) and compared with non-mutagenic plants (control treatment). The cultures were incubated at 25 ± 2 °C and 16 h day^{-1} illumination. Mutated and non-mutated plants of the three cultivars were transferred to the greenhouse and data were taken on the number of minitubers, diameter and their weight after 90 days of planting. Random Amplified Polymorphic DNA (RAPD) technique was applied to detect genetic variations using 6 random primers (A8, A10, C8, C15, H16 and S12).The results showed that the percentage of mortality (LD_{50}%) of stem cuttings of the cultivars (Arnova, Provento and Emma) was 30 minutes for mutation with sodium azide at 1 mM concentration The results also showed the significant deference between the cultivars in their response to the mutation with sodium azide at different periods of time in the phenotypic traits of vegetative and root system. The six primers distinguished a number of bands with different molecular weights in mutated plants and their absence in non-mutated plants within the same cultivar. C15 primer was distinguished 3 bands in mutagenic plants of Arnova cultivar and 5 bands of Provento cultivar. While the primers A8 and S12 distinguished only one band with a molecular weight of 1000 base pair in the mutated of Arnova and Provento, as well as the primer A10 distinguished one band in the cultivar Provento and two bands in the cultivar Emma.

Keywords: Potato. Sodium azide. RAPD. $LD_{50\%}$. Minitubers.

Introduction

Potato (*Solanum tuberosum* L.) is an important vegetable crop in the world in terms of production and cultivated area. Potatoes are grown in about 150 countries in the world (Basera et al., 2018). FAO statistical ,2020 indicate that the world's productivity of the potato crop has reached 368 million tons, with a cultivated area of 17.5 million hectares. While the productivity in Iraq for the same period amounted to 294 million and a cultivated area amounted to 18,789 ha..The potato crop ranks second in terms of human consumption because

it is the main food for many people in the world and is the most important food crop other than cereals in the world (MORIAS et al., 2018 Bamberg et al., 2016; Nikitin et al., 2018), It is a rich source of many nutrients as well as containing a high percentage of starch and protein. Based on the principle of the global food crisis and the thinning of suitable spaces and climatic changes, it was necessary to search for methods to derive new genotypes from agricultural crops that would be able to adapt and produce under different stress conditions and improve their phenotypic traits. Therefore, it is necessary to follow all new techniques in order to propagate the crop and increase its productivity As a plant tissue culture technique (Al-Salihi, 1994) and *in vitro* mutagenesis to obtain the required genetic variations (Al-Sumaidai, 2016). Physical and chemical mutagenesis has been used in many studies, whether for potato or other crops towards obtaining genotypes with quantitative and qualitative traits or tolerant of environmental stresses (Gómez, et al. 2018), It is possible to expand the base of genetic variations by employing plant tissue culture technique and *in vitro* mutagenesis to produce genotypes that have some of these traits (Ahmad et al., 2010 Al Hussaini, 2016). The use of sodium azide (NaN3) as a chemical mutagen has proven its efficiency in causing genetic variation or improving morphological traits of a wide range of plants (Adebola., 2013; Al-Kaabi and Akharsen, 2015), and for developing resistance in sensitive crops to improve yield and qualitative traits against pathogens ((Olawuyi and Okoli, 2017). Whereas sodium azide causes a point mutation in the plant genome, producing a protein that has a different function compared to the non-mutated plant where the mutated plant can survive under unfavorable conditions (Khan et al., 2009). Sodium azide was used to mutagenesis different parts of the plant such as seeds (Szarejko, 2017) and vegetative tissues (Gómez, 2019), and the concentrations are often in the range of 0.5-4 mM. Ahmed et al., 2010 studied the effect of different concentrations of sodium azide (0.1 , 0.2, 0.3, 0.4 and 0.5 mM) in the regeneration stage from callus that exposed to a mutagenesis factor for three cultivars of potatoes (Cardinal, Diamant and Desiree) which induced from different explants (microtubers, root, leaf, Nodes, stem cuttings) Where they obtained a mutated plant from callus which induced from the leaf of Desiree cultivar exposed to the mutagenesis factor at a concentration of 0.1 mM and another of callus induced from the leaf of Cardinal cultivar at a concentration of 0.4 mM. In a study conducted of Divanli-Turkan et al., 2006 on the pea plant by exposing the seeds of four cultivars of peas (Winner, Karin, Sprinter and Bolero) to different concentrations of sodium azide (0.001, 0.002, 0.003, 0.004, 0.005 molar) for an hour and grown in the medium contains different growth regulators (BA and TDZ),The results of LD50 showed that 0.001M NaN3 concentration was most suitable for mutagenicity and was not fatal. As for Gomez et al., 2019, they indicated that the 50 % reduction rate in the *in vitro* multiplication of sugarcane explants was at 0.23 mM sodium azide, suggesting that this concentration is very suitable for the method of vegetative multiplication using the temporary immersion system using the bioreactor (TIP) Temporary. emersion bioreactor thus producing agriculturally useful mutated.

The successes achieved by genetic engineering with the development of biology molecular have contributed to the emergence of many techniques suitable for the molecular analysis of genetic material, including the Random Amplify Polymorphic DNA (RAPD) indicators Which depends on the Polymerase Chain Reaction (PCR) technique, which is characterized by its speed and simplicity and does not require a large amount of DNA, in addition to the possibility of applying it to large-sized genetic populations with its use of random primers that allow covering different regions of the genomes of the studied individuals (Al-Zaidi et al., 2016). These indicators have been used in many research and studies (Al-Tikriti, 2002; Al-Hussaini, 2016).Therefore, this research aims to determine the optimum time period for the effectiveness of the chemical mutagen Sodium Azide in influencing the morphological traits of the vegetative and root system of three potato cultivars (Arnova, Provento, Emma) and the possibility of obtaining mutated plants with desirable traits that can be entered later in the *in vitro* screening and selection plants with desirable traits.

Materials and methods

Tissue culture experiments, DNA extraction and polymerase reactions were conducted in the Molecular Biology Laboratory - Genetic Engineering Department - Food and Biotechnology Center in the Agricultural Research Department / Ministry of Science and Technology 2021/2022. In the experiment, three cultivars of potato approved for cultivation in Iraq were used, namely Arnova, Provento and Emma. The tubers were incubated in the laboratory at a temperature of +25°C - 2°C with indirect lighting for two weeks to break the dormancy and stimulate the growth of vegetative buds. Then the apical meristem were isolated from vegetative growths with a length of 0.1-0.3 mm with a pair of leaf primordia and cultured in test tubes containing 15 ml of MS medium (Murashige and Skooge, 1962) imported by the Indian company H Media (4.4 g l^{-1}) with addition Sugar (30 g L^{-1}) and agar (6-7 g L^{-1}) for solidification of the medium. The cultures were incubated at a temperature of 25 ± 2 and 16 hours of illumination on day^{-1}. After three weeks, the *in vitro* propagated plants were cut into stem cuttings 1-2 cm long, containing 1-2 nodes, and planted in test tubes containing 15 ml of the previous medium and incubated under the same conditions as then re-cultures every month to obtain the numbers required for subsequent experiments (Fig. 1).

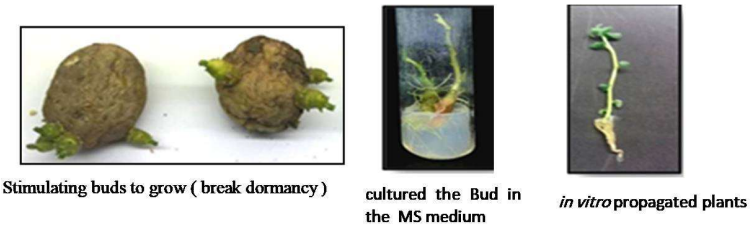


Figure (1). Initiation stage of three cultivars of potato cultured in MS medium.

Mutagenesis with sodium azide (NaN3).

In vitro Plants mutated with sodium azide at a concentration of 1 mM, which was prepared by dissolving it in phosphate solution (PVR) prepared from (Citric acid (0.1 M) and Na2Hp4 (2.0 M) at pH (3.0), mutated for different periods of time (5,10. 15 and 30 minutes. Mutated and non-mutated plants were cut to stem cuttings (between the nodes with a length of 1-1.5 cm) and planted in test tubes containing the available MS medium for micropropagation with 10 replicates for each treatment (stem cuttings Rep^{-1}). The cultures were incubated under the previous conditions (Figure 2). The mutation time period with sodium azide was determined based on the time period that caused mortality percentage (50%, LD_{50}) of the stem cuttings compared to the non-mutated (comparison treatment). Data were taken on the phenotypic traits of the vegetative growth (plant height, number of shoots, leaves and nodes) and roots growth (number of roots and their length).

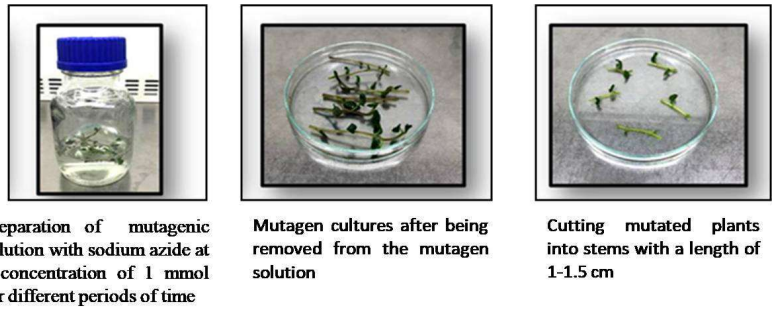


Figure (2). Mutation steps with Sodium azide at concentration 1 mM for different periods of time for *in vitro* propagated plants of tthree scultivars of potato..

Field performance

The mutated plants (optimum time period) and non-mutated plants of the three studied cultivars were transferred to the greenhouse after acclimatization for two weeks under laboratory conditions (temperature 25 °C ± 2 and indirect lighting,). They were planted in nylon bags of 1 kg capacity, with 5 replicates for each cultivar, then covered with plastic sheets to provide moisture for two weeks, covers were lifted, then watered and fertilized according to the fertilizer recommendation. Data of number (tuber plant-1), diameter (cm) and weight (g) of the minitubers were taken after 90 days.

Statistical analysis

Factorial experiments were conducted using Completely Randomized Design (CRD) (10 replicates for *in vitro* culture and 3 replicates for field performance). The data were statistically analyzed using the statistical program Genstat. Means were compared using the Least Significant Difference (LSD) test at a probability level of 5%.

Random Amplified Polymorphic DNA (RAPD) Technique

Isolation of total DNA from leaves of mutated (optimal) and non-mutated plants of the three cultivars of potato as shown in Table (1) using CTAB in the manner described by Borges et al., 2009 with some modifications.

Table 1. Plant samples and their numbers in the multiplication stage

No.	plant samples
1	Arnova Non-mutated plants
2	Arnova Mutated plants
3	Provento Non-mutated plants
4	Provento Mutated plants
5	Emma Non-mutated plants
6	Emma Mutated plants

The DNA concentrations were measured and their purity was estimated by the (Nanodrop) DNA. The reaction was conducted using the PCR® AccuPower kit PreMix using 6 random primers as shown in Table (2). The samples were placed in the Thermal Polymerase chain Reaction apparatus (PCR), and the serial program was carried out, which starts with the initial denaturation of the DNA strand, one cycle for 4 minutes at a temperature of 94°C. Denaturation 35° cycles multiply, Each cycle includes one minute at 94 °C, DNA template primers annealing for one minute at 36 °C, then elongation for one minute at 72 °C with a final cycle of 10 minutes at 72 °C for final elongation). The reaction products were electrophones with Ladder DNA 100 bp (Korea Pioneer Company) via a garose gel (1% concentration and stained with 30 ng ethidium bromide) and in the presence of TBE buffer (1x) in a electrophoresis for 0.45 hours. DNA was vitualed with ultraviolet rays. The number of bands was calculated and their molecular weights were determined using the Photocapt computer program. The polymorphism percentage of primer, the percentage of the discriminative ability of each primer, and the percentage of efficiency of each primer, were calculated using the following equations (Al-Judy and Majeed, 2013):

Polymorphism % = (the number of polymorphic bands of the random primer / the total number of bands of the same primer) x 100

Discriminative ability % = (the number of polymorphic bands of the random primer / the number of polymorphic bands of the all random primers) x 100

Efficiency of each primer % = (the total number bands of random primer / the total number bands of all the random primers) x 100

Table (2) Random primers with their sequences

No.	primer name	3 ⟶ 5 primers sequence
1	OP-A8	GTGACGTAGG
2	OP- A10	GTGATCGCAG
3	OP- C8	TGGACCGGTG
4	OP- C15	GACGGATCAG
5	OP- H16	TCTCAGCTGG
6	OP-S12	CTGGGTGAGT

Results and Discussion

Determining the optimum duration of mutagenesis with sodium azide NaN3

It is noted from the results that the percentage of mortality (LD_{50}%) of stem cuttings of the cultivars (Arnova, Provento and Emma) increased with the increase of the time of mutagenesis with sodium azide at concentration of 1mM (Table 3 and Figure 3) compared with non-mutated plants and the treat plants with sodium azide at 10 and 15 minutes. Based on to the previous studies, the optimal dose that gives the 30- 50% morality or decreasing in growth is a criterion for the optimal dose at which a sufficient amount of plants grow later, which gives viable seeds that produce non-sterile mutated plants (Al-Tikriti, 2002; van Harten, 1998; Divanli- turkan 2006). The optimum time period that gave 50% mortality percentage of the stem cuttings was determined, which was 30 minutes. This result was agreement with Gomez and Akhrozun, 2019 who indicated the importance of determining the optimal dose in producing agriculturally beneficial mutations.

Table 3. Effect of mutagenic time with sodium azide (NaN3)) at a concentration of 1 mM on mortality percentage (%) of stem cuttings of the three cultivars of potato in vitro

Time (minute)	**mortality percentage (%) Cultivars**			**Average**
	Arnova	**Provento**	**Emma**	
0.0	100	100	100	**100**
10.0	100	100	100	**100**
20.0	90	90	90	**90**
30.0	50	50	50	**50**
Average	**85**	**85**	**85**	
L.D.S 0.05				
Cultivars		Time		cultivars x Time
N.S		15.65		27.10

Arnova

Provento

Emma

Figure 3. The response of the stem cuttings of cultivars to mutagenesis with sodium azide at a concentration of 1mM for a period of 30 minutes.

Effect of mutagenic time with sodium azide (NaN3) on the phenotypic traits of the shoot and root system of three potato cultivars *in vitro*.

It is clear from the results of the statistical analysis Table (4) that the treatments and their interactions had a significant effect on the plant height trait. The results showed a decrease in the plant height with increasing the time period of mutation with sodium azide, where the plant height average decreased from 11.12 cm at the time period (0.0) minutes to 3.75 cm At the time of (30) minutes. The results of the same table also showed that there were significant differences between the cultivars in this trait, as the Arnova cultivar plants gave the highest rate of plant height (88.7 cm) and did not differ significantly from the plants of the cultivar Emma, while the lowest rate of plant height was achieved for the Provento cultivar, with an average of 86.6 cm. The reason for the difference in plant height between cultivars may be due to genetic reasons related to the same cultivar. Moreover, the interaction between the cultivars and the time period of mutagen was affected on this trait, and that the highest rate of plant height was achieved for the plants of the cultivar Emma in the control treatment (non mutated) that reached 60.11 cm. While the treatment (Arnova cultivar + 30 minutes) gave the lowest average plant height was 55.3 cm. As for the number of shoots, the results showed that there were significant differences for the duration of soaking the plants with the mutagen sodium azide (Table 4). The plants that were soaked with sodium azide for 10 minutes gave the highest average number of shoots 1 shoot. plant^{-1}, while the time period of 30 minutes failed to give shoots. The results in the same table also indicate that there are significant differences between the cultivars in terms of the number of shoots, and the Provento cultivar was gave higher number of shoot (0.60 shoot.plant^{-1}) than others . As for the interaction, the results showed that was significant, where the Provento cultivar excelled when soaked with mutagen sodium azide for 10 minutes giving 1.60 leaf. plant $^{-1}$ compared to other treatments that achieved low averages number of shoots. The results of Table 4 showed excelled of the

time period of soaking plants with sodium azide for 10 minutes by giving the highest average number of leaves compared to others periods (15 and 30 minutes) that reached 11.40 leaf.plant^{-1}. Which not differ significantly from control treatment (non-mutated) (10.37 leaf.plant^{-1}), while the lowest average number of leaves observed in the period of time 30 minutes was 4.37 leaf.plant^{-1}. The cultivars had significant differences in the number of leaves (Table 4). The results of the same table also showed that there was a significant effect of the interaction. that Provento cultivar excelled when treated with sodium azide for 10 minutes with leaves number 12.70 leaf.plant^{-1}, while the lowest number of leaves was 3.60 leaf.plant^{-1} in Arnova cultivar when soaked with sodium azide for 30 minutes. The results of the statistical analysis in Table 4 indicate that the period of time for mutation affected on average number of nodes, where it decreased significantly from 8.17 node.plant^{-1} to 3.07 node. plant^{-1}. The results of the same table also showed that there were no significant differences between cultivars in this trait. The interaction had a significant effect on the average number of nodes, where the highest rate was 9.20 nodes. plant $^{-1}$ of Provento cultivar when soaked for 10 minutes .While the lowest average number of nodes observed Arnova which soaked for 30 minutes (2.40 nodes .plant^{-1}).

Table 4. Effect of Mutagenesis with sodium azide(NaN3)) at a concentration of 1mM on the phenotypic traits of the shoots of three potato cultivars in vitro.

time period	**plant height (cm)**			**Average**
	Cultivars			
	Arnova	**Provento**	**Emma**	
0.0	11.20	10.55	11.60	**11.12**
10.0	10.35	6.50	7.65	**8.17**
15.0	6.40	6.35	6.36	**6.33**
30.0	3.55	4.05	3.65	**3.75**
average	**7.88**	**6.86**	**7.29**	
		L.D.S 0.05		
cultivars		**time period**	**Cultivar sx time period**	
1.29		**1.49**	**2.59**	
		Number of shoots (shoot.plant^{-1})		
0.0	0.00	0.30	0.20	**0.17**
10.0	0.60	1.60	0.80	**1.00**
15.0	0.30	0.50	0.60	**0.47**
30.0	0.00	0.00	0.00	**0.00**
average	**0.23**	**0.60**	**0.40**	
		L.D.S 0.05		
cultivars		**time period**	**Cultivars x time period**	
0.32		**0.37**	**0.63**	
		Number of leaves. plant $^{-1}$		

0.0	10.50	10.60	10.00	**10.37**
10.0	9.60	12.70	11.90	**11.40**
15.0	9.30	10.00	7.40	**8.90**
30.0	5.00	4.50	3.60	**4.37**
average	**8.60**	**9.45**	**8.22**	
		L.D.S 0.05		
cultivars		**time period**	**Cultivars x time period**	
NS		**1.81**	**3.13**	
		Number of node(node .plant^{-1})		
0.0	7.20	7.90	6.90	**7.33**
10.0	8.50	9.20	6.80	**8.17**
15.0	5.20	c7.00	6.60	**6.27**
30.0	2.40	2.90	3.90	**3.07**
average	**5.82**	**6.75**	**6.05**	
		L.D.S 0.05		
cultivars		**time period**	**Cultivars x time period**	
NS		**1.29**	**2.23**	

With regard to the effect of sodium azide on the phenotypic traits of the root system, it is clear from Table (5) that the treatments and their interactions had a significant effect on the number of roots. The study showed that increasing the time period of treatment with sodium azide led to a decrease in the number of roots, especially the time period (30 minutes, which gave the lowest average number of roots reached 4.77 root plant^{-1} Compared to the control treatment (non-mutated), which had the highest average number of roots 14.07 root plant^{-1}. The results of the same table also showed that there were significant differences between the cultivars in this trait, Emma cultivar excelled than Provento by giving the highest number of roots (9.93 root plant^{-1}), which not differ significantly from Arnova cultivar (9.23 root plant^{-1}). Whereas, the Provento cultivar gave the lowest number of roots (7.73 root plant^{-1}). The reason for this may be due to the genetic differences between the cultivars. The interaction between the cultivars and the time period had a significant effect on the number of roots, where the cultivar Emma at control treatment (non- mutated) gave the highest number of roots was 18 root plant^{-1} compared to the treatment (Arnova + 30 minutes) which gaved the lowest number of roots (3.50 root plant^{-1}). The time period of exposing plants with sodium azide significantly effected on the length of roots (Table 5), as control treatment (non-mutated) gave the highest length of the root (9.90 cm), while the lowest root length was achieved at the time period 30 minutes (4.03 cm). Also Results showed that the cultivars no significant differences between them in this trait. Statistical analysis (Table 5) showed that there were significant effect of the interaction between the cultivars and the time period, the treatment (Arnova + non- mutated) was excelled in giving the highest length of root (10 cm) compared to the other interaction treatments which excelled than treatment (Arnova + duration of 30 minutes) that achieved the

lowest average of root length of 2.90 cm. It appears from the results that increased the duration of exposure to the mutagen negatively affected 0n the phenotypic traits compared with non-exposed to the mutagen. This may be explained by the fact in vitro propagated plants grow in a nutrient medium supplemented with Vitamins, growth regulators and sucrose, which are necessary in the continuity of growth, that when the plant is exposed to any stress. As with both types of mutation, the effectiveness of plant growth may decrease under the influence of this stress, thus negatively affecting on the phenotypic traits. or it may be explained that the effect of sodium azide interaction with biochemical and genetic processes within the plant (Ahmed et al., 2010).

Table 5. Effect of mutagenicity with sodium azide (NaN3) at a concentration of 1 mM on the number and length of roots for three cultivars of potato in vitro.

Time period	Number of roots (root .plant-1)			Average
	Cultivars			
	Arnova	Provento	Emma	
0.0	13.00	11.20	18.00	**14.07**
10.0	12.30	7.60	9.00	**9.63**
15.0	8.10	7.40	6.60	**7.37**
30.0	3.50	4.70	6.10	**4.77**
average	9.23	7.73	9.93	
L.D.S 0.05				
Cultivars 1.88		**time period 2.17**		**Cultivars x time period 3.75**
root length (cm)				
0.0	10.00	9.90	9.80	**9.90**
10.0	8.80	8.90	6.50	**8.07**
15.0	6.60	7.80	6.30	**6.90**
30.0	2.90	4.80	4.40	**4.03**
average	7.08	7.85	6.75	
L.D.S 0.05				
Cultivars N.S		**time period 1.42**		**Cultivars x time period 2.46**

Morphological traits of mini tubes grown in a greenhouse.

Most plants growing *in vitro* are characterized by a different metabolism process than those growing in the field, where the nutrient medium provides a source of carbon, growth regulators, High humidity with low levels of carbon dioxide and light, which stimulates growth and shifts plant nutrition from autotrophic to discarded (Chandra et al., 2010), when transferred to the field, plants must acclimate to those conditions, which are stressful in multiple ways (Teixeira et al., 2017).The results of the statistical analysis in Table 6 (Figure 4) indicate that there is no significant difference between mutated and non-mutated plants in

the average number of tubers. While, results showed significant differences in the average of the diameter and weight of minitubers from *in vitro* non-mutated and mutated plants with sodium azide for three cultivars. Whereas, the non-mutagenic plants of the cultivar Emma excelled with the highest mean of tuber diameter of 6.11 cm, respectively, which not differ significantly from the two cultivars, Provento and Emma (non - mutated plants) reached 5.49 cm and 4.22 cm, respectively. The mutated and non-mutated plants also differed significantly in the average weight of minittubers, that the Provento mutated plants were excelled in this trait and gave the highest mean weight of 1.077 gm, which did not differ significantly from the rest of the plants except for the Arnova non-mutated plants which gave the lowest mean weight (0.180 gm).

Table (6). Phenotypic traits of Minitubers of *in vitro* mutated and non- mutated plants of three cultivars of Potato cultured in field.

Plants	Number (tuber plant^{-1})	Diameter (cm)	Weight (gm)
Arnova non-mutated plants	2.33	0.72	0.180
Provento non-mutated plants	2.67	2.49	0.700
Emma non-mutated plants	2.33	6.11	1.025
Arnova-mutated plants	2.67	1.12	0.521
Provento - mutated plants	2.00	5.49	1.077
Emma - mutated plants	2.00	4.22	0.483
L.S.D. 0.05			
Number	**Diameter**	**Weight**	
NS	2.513	0.735	

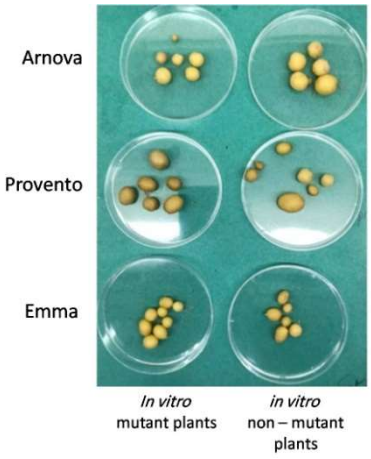


Figure 4. Production Minitubers from *In vitro* non – mutated and mutated plants with sodium azide (1mM for 30 minute) for three cultivars of Potato cultivars after 90 days under green house condition.

Random Amplify Polymorphic DNA (RAPD-DNA)

Table 7. Concentration and purity of DNA extracted from leaves of different cultivars of potato mutated with sodium azide (NaN3).

genotype	Non- mutated plants		Mutated plants	
	Purity	DNA concentration (ng µl-1)	Purity	DNA concentration (ng µl-1)
Arnova	2.00	254.9	2.00	254.9
Provento	2.00	206.3	2.00	206.3
Emma	2.01	270.4	2.01	270.4

The DNA concentration of the plant samples (mutated and non-mutated) ranged between 206.4 and 458.4 ng μmol^{-1}, with a purity of between 2.00 and 2.01 for the samples (Table 7). The isolated amounts of DNA and the degree of purity in the extraction stage were appropriate and had a positive impact on the subsequent steps.

The PCR-RAPD technique was used with the presence of 6 random primers, which depended on the method of analyzing the results of the study of genetic variations on the presence or absence of the bands resulting from the multiplication of certain pieces of the genome of the samples used and on the molecular weights of those bands that depend on the number and complementary positions of the primer sequences on the template DNA, they were neglected very light bands (Swoboda and Bhalla, 1997).

Polymorphism, primer efficiency and their discriminative ability

All primers have proven their effectiveness in giving polymorphism between the studied sites, and this may be due to the fact that the primer looking for similar regions in the DNA strand, and when it finds them, the product is doubled. The use of these primers resulted in a number of bands with a total of 52 bands of which 31 bands gave a polymorphism (Table 8), and the primers varied in the number of bands that the lowest number of bands sites was in the C8 primer, while the highest number of bands sites appeared in the primer C15 (11 sites). As for calculating the percentage of polymorphism, efficiency and discriminating ability for each primer, it was noted that the highest percentage of polymorphism was 90.91% was in the primer C15. While the lowest percentage of polymorphism it was 44.44% in the primer S12 due to the number of its polymorphic bands is almost half of the total number of its bands. The highest efficiency and highest discriminating ability (21.15% , 32.26%) were recorded in the primer C15.

Table 8. Polymorphism, efficiency and their discriminative ability of the primers

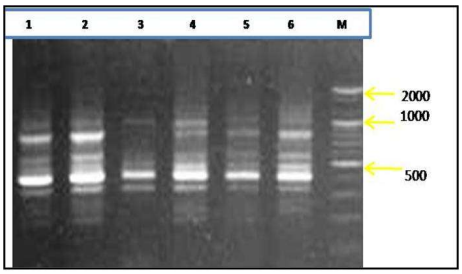
primer symbol	Total number	The number of	polymorphism primers %	primers efficiency %	Discriminative ability of primer %

	of bands	polymorphism bands			
A8	8	5	62.50	15.38	16.13
A10	10	5	50.00	19.23	16.13
C8	6	1	16.67	11.53	3.23
C15	11	10	90.91	21.15	32.26
H16	8	6	75.00	15.38	19.35
S12	9	4	44.44	17.31	12.90
Summation	**52**	**31**			

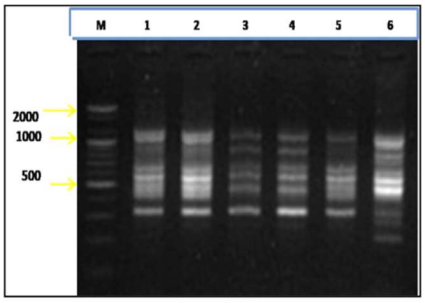
The results of the RAPD technique for the six primers showed clear differences between mutated and non-mutated plants of the same cultivar, where the results of RAPD reactions varied according to the primer used. The average number of bands was 52 bands according to the DNA of the mutated and non-mutated plants with molecular weights ranged 200-1900 base pairs. As for polymorphism bands which are essential in estimating the genetic varations between cultivars it reached 31 bands (Table 8). The difference in the number of bands may be due to the different nitrogen bases in the genotypes (Al-Zaydi, 2016). The primers distinguished a number of bands at different molecular weights in mutated plants and their absence in non- mutated plants within the same cultivar (Table 9 Figures 6, 7 and 8), which is fully focused on in this study, The C15 primer was characterized by its ability to identify a number of bands of different molecular weights in mutated plants as it distinguished 3 bands in mutated of the cultivar Arnova 450, 400 and 250 bp and 5 bands for the Provento cultivar (1800, 1000, 550, 350 and 250 bp). While the primers A8 and S12 distinguished only one band with molecular weight 1000 bp in the mutated plants of Arnova and Provento, as well as the primer A10 distinguished one band with molecular weight 250 bp in the cultivar Provento and two bands 900 and 250 bp in the cultivar Emma. These results were consistent with other studies that indicated the excelled of RAPD technology and its ability to analyze genetic variations between plant species, in addition to its ability to distinguish between cultivars of one species (Al-Zaidi et al., 2010). It is also a powerful tool in molecular genetic analysis and detection of mutations in the genotypes of some crops such as rice (Babaei et al., 2011). It can be said that the products of the reaction showed a number of bands that were able to distinguish the genetic variations that appeared between plants exposed to the mutagenic factor of sodium azide from those of non-mutated plants. This may be explained to the mechanism of sodium azide by production of an azide compound that interaction with the DNA of the cell nucleus. This causes a point mutation, suggesting that SA replaces GC with AT, subsequently causing some changes in amino acid production (AL-Qurainy and Khan, 2009) .Also with some studies (Salim et al., 2009, Dubey et al., 2017) indicated that the mechanism of action of sodium azide is based on the production of an organic metabolite (β-azidoalanine [N3–CH2–CH(NH2)–COOH]) leading to chromosomal aberrations at lower rates compared to other substances.

Table (9) the primers that distinguished the plant samples and the molecular weight of each distinct bands

Plant samples	primer symbol	Molecular weight of the Unique and visible bands (bp)
Arnova non-mutated plants	A8	250 ، 500
	A10	900
	C15	500 ،550
Arnova mutated plants	A8	1000
	C15	400،250 ،450
Provento non-mutated plants	A8	200 ، 250 ،،600،500
Provento mutated plants	A10	250
	C15	260 ،350 ،550 ،1000 ،1800
	S12	1000
Emma non-mutated plants	A8	250
	C8	450
	H16	300 ،350
Emma mutated plants	A10	250 ،900
	H16	500 ، 600 ،100



primer A8



primer A10

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1000	0	1	1	1	0	0
2	800	1	1	1	1	1	1
3	600	1	1	1	0	1	1
4	500	1	0	1	0	1	1
5	400	1	1	1	1	1	1
6	350	1	1	1	1	1	1
7	250	1	0	1	0	1	0
8	200	0	0	1	0	1	1

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1200	1	1	1	0	0	0
2	1000	1	1	1	1	1	1
3	900	1	0	0	1	0	1
4	700	1	1	1	1	1	1
5	600	1	1	1	1	1	1
6	500	1	1	1	1	1	1
7	400	1	1	1	0	0	0
8	300	1	1	1	1	1	1
9	250	0	0	0	1	0	1
10	200	0	0	0	0	0	1

0 : Absent band 1: Presence band

Figure 7 The PCR products with primers A8 and A10 with their molecular weights for potato plant samples which electrophoresed on 1% agrose gel.

M: DNA ladder 100 bp M: DNA ladder 100 bp

1: Arnova non-mutated plants

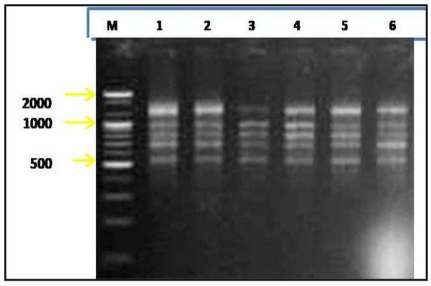
2: Arnova mutated plants

3: Provento non-mutated plants

4: Provento mutated plants

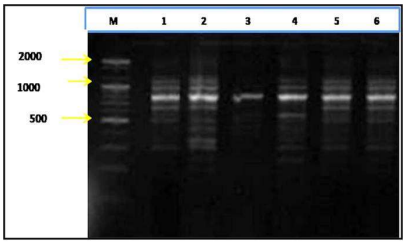
5: Emma non-mutated plants

6: Emma mutated plants



primer C8

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1800	1	1	1	1	1	1
2	1000	1	1	1	1	1	1
3	800	1	1	1	1	1	1
4	700	1	1	1	1	1	1
5	500	1	1	1	1	1	1
6	450	0	0	0	0	1	0



primer C15

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1800	1	1	0	1	1	1
2	1000	1	1	0	1	1	1
3	800	1	1	1	1	1	1
4	700	1	1	0	0	1	1
5	600	1	1	0	0	1	1
6	550	1	0	0	1	0	0
7	500	1	0	0	0	1	1
8	450	0	1	0	0	0	0
9	400	0	1	0	0	0	0
10	350	1	1	0	1	1	1
11	250	0	1	0	1	0	0

0 : Absent band 1: Presence band

Figure 8 The PCR products with primers A8 and A10 with their molecular weights for potato plant samples which electrophoresed on 1% agrose gel.

M: DNA ladder 100 bp M: DNA ladder 100 bp

1: Arnova non-mutated plants

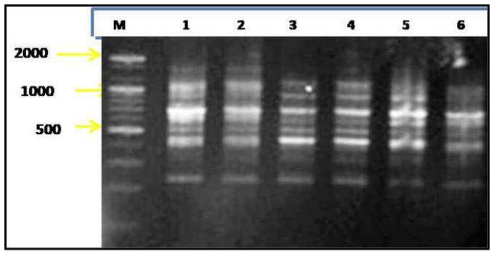
2: Arnova mutated plants

3: Provento non-mutated plants

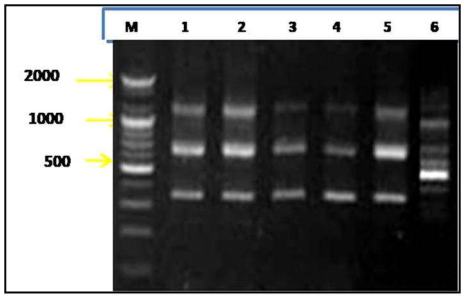
4: Provento mutated plants

5: Emma non-mutated plants

6: Emma mutated plants



Primer S12



Primer H16

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1900	1	1	0	0	0	0
2	1100	1	1	1	1	0	0
3	1000	0	0	0	1	1	1
4	700	1	1	1	1	1	1
5	600	1	1	1	1	1	1
6	500	1	1	1	1	1	1
7	400	1	1	1	1	1	1
8	350	0	0	1	1	0	0
9	250	1	1	1	1	1	1

NO.	Molecular Weight (bp)	1	2	3	4	5	6
1	1100	1	1	1	1	1	1
2	1000	0	0	0	0	0	1
3	700	1	1	1	1	1	1
4	600	0	0	0	0	0	1
5	500	0	0	0	0	0	1
6	400	0	0	0	0	0	1
7	350	1	1	1	1	1	0
8	300	1	1	1	1	1	0

0 : Absent band 1: Presence band

Figure 8 The PCR products with primers S12 and H16 with their molecular weights for potato plant samples which electrophoresed on 1% agrose gel.

M: DNA ladder 100 bp M: DNA ladder 100 bp

1: Arnova non-mutated plants

2: Arnova mutated plants

3: Provento non-mutated plants

4: Provento mutated plants

5: Emma non-mutated plants

6: Emma mutated plants

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